

# USING ALTACH 5EC TO TREAT THE ROOTS OF *ALLIUM FISTULOSUM* L. CONTRIBUTING TO IMPROVING THE EFFECTIVENESS OF THE “CHROMOSOMAL STRUCTURAL MUTATIONS” EXERCISES IN GENETICS - EVOLUTION AT BIOLOGY DEPARTMENT, VINH UNIVERSITY

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## ARTICLE INFORMATION ABSTRACT

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In this study, a commercial insecticide, Altach 5EC, has been used to investigate its cytotoxic effects on chromosomes and mitosis of cells in the root tip of *Allium fistulosum* L. The result indicated that Altach 5EC which containing two different concentrations of alpha-cypermethrin (0,047 mg/ml and 0,062 mg/ml) were used to treat the root tips for 8 hours led to a decrease in the mitosis index compared to the negative control. Especially, various types of chromosomal mutations were observed such as broken chromosomes, chromosomal bridges, and slow-moving chromosomes. At the same time, numerous disturbances of mitosis were observed, which added the data for the practice lesson of *Mitosis*. These disturbances included sticky chromosomes, metaphase disturbance, fault polarization in anaphase and telophase, and sticky metaphase. The study revealed that Altach 5EC is a suitable commercial insecticide for effectively treating the roots of *Allium fistulosum* L. in both the “Chromosomal Structural Mutations” and “Mitosis” lessons within Genetics - Evolution subject at Biology Department, Vinh University.

**Keywords:** *Allium fistulosum* L.; chromosome mutations; commercial insecticide; index mitosis.

## 1. Introduction

“Chromosomal Structural Mutations” is a practical lesson within Genetics- Evolution Subject for students of biology at Vinh University. To ensure effective implementation of this practice lesson and to develop practical skills for students, in addition to guiding in preparing well the chemicals for making slides and tools, orientation on how to prepare satisfactory slides for practice is an extremely important factor.

Chromosomal mutations can arise in living cells due to both physical and chemical factors. Research in Vietnam and the world have demonstrated that gamma rays [1], X-rays [7], mobile phone radiation [5], temperature (heat shock) [1], and chemical factors [8] can cause chromosomal mutations and disorder cellular division. However, the use of most of these factors in the preparation of materials for experiments at the university needs appropriate equipment such as radiation

shielding systems to ensure the safety and health of the technician and students who are preparing the samples, or a heat shock incubator, the purchase of toxic and costly chemicals like EMS (ethyl methanesulfonate), etc. Therefore, it is crucial to research and select cost-effective, safe, easily available, and time-appropriate agents for laboratory experiments.

Many studies in the world have indicated that certain types of insecticides that are used in agriculture can cause genetic disorders in cells of plant species [2], [3], [6], [9], [11], [18]. Among them, *Allium* species are the most extensively used objects for investigating the effects of chemicals on chromosomal mutations and cell mitosis [3], [6], [11]. In fact, *Allium* species are ideals for researching the impact of toxins on chromosomes and cells because of its numerous advantages: (1) Its growth is very sensitive to pollutants; (2) The mitotic phases are very clear; (3) Stable chromosome number and stable karyotype; (4) Diversity of chromosome morphology; (5) Clear and fast response to the genotoxic substances; (6) Spontaneous chromosomal damages occur rarely. Thus, in this study, *Allium fistulosum* L. was used to investigate the effects of Altach 5EC - a widely sold insecticide in Vietnam on chromosomal mutations and mitotic index during cell division, applying it as a chromosomal mutagen in preparing samples for the lesson “Chromosome Structural Mutations” within Genetics - Evolution subject.

## **2. Materials and methods**

### **2.1. Test samples**

The test samples used in this study are the onion roots of *Allium fistulosum* L., which is extremely popular in Nghe An as well as in Vietnam. Roots were facilitated to grow and harvested from onion “bulbs” purchased from a farmer household in Hung Loc commune, Vinh city, Nghean province. Selected onion “bulbs” of the same color are uniform in size and healthy.

### **2.2. Chemicals and equipment**

Chemicals used in this study include: (1) Altach 5EC 10 ml package, a commercial product produced by Insecticide India Limited (India), containing the active ingredient alpha -cypermethrin at concentration 50 g/l (as a chromosomal mutagenic agent) and can effectively eliminate various pests such as worms, sucking, thrips damage rice, aphids, leaf borers, bugs .... (2) ethanol and glacial acetic acid for cell fixation, and (3) carmin acetic for staining chromosomes.

In the study, equipment was used including (1) Optical microscope with camera, and (2) an incubator.

### **2.3. Methods**

#### **2.3.1. Sample preparation**

The loose outer scales of onion bulbs were removed, and old roots were scraped to expose the primordial roots before immersed into warm water at 60°C for 3 hours. Then, all the bulbs are divided into three equal parts (per part consisting of 10 bulbs) and grown

in clean sand until new roots reach 0,5-1,0 cm. One part was used as negative control, the other two parts are treated by diluted Altach 5EC with two different concentrations of alpha- cypermethrin 0,047 mg/ml and 0,062 mg/ml (as recommended by the manufacturer) for 8 hours (a convenient time for sample preparation and used by many other studies).

### 2.3.2. Temporary slide preparation

New onion roots, approximately 0,5-2,0 cm in size, straight, white, fresh, and intact were gently washed in clean water, dried, and then detached. The assay was performed based on the method described by Matsumoto *et al* [15] as follow: (1) The roots were fixed in a prepared solution of 3:1 (v/v) alcohol-glacial acetic acid for 24 hours at 25°C; (2) The roots were treated with 1 N-HCl for 5 minutes to hydrolyse and soften the tissues; (3) Roots tips were washed with distilled water to remove HCl; (4) The roots were stained with acetocarmine 1% for 10 min at 30°C; Finally, each root was leted on a slide to creat temporary slides.

### 2.3.3. Observation of temporary slides and data analysis

Observing, counting, and analyzing cells on temporary slides is based on the method proposed by Rank and Nielsen [17].

Step 1: (*Observation*): The temporary slides were viewed under a light microscope using the 20X, 40X and 60X objective lenses. Then, the most representative examples of mitosis cell regions, various types of chromosomal mutations, or disturbances of mitosis were identified and photographed for further analysis in step 2. To ensure the reliability of the study, at least 30 onion roots were prepared as temporary slides for each active ingredient concentration and negative control. At the same time, to avoid duplication during the cell analysis, the cell regions are observated from left to right, from top to bottom, without repetition.

Step2: (*Counting and analysis cells*): A total of 1000 cells from each concentration and negative control were selected for analysis, and determining the number of cells in prophase, metaphase, anaphase, and telophase, as well as the number of cells with chromosomal mutations or disturbances of mitosis. The number of cells was counted using Paint software and analyzed based on obtained images. Specifically, the cells in the images were counted from right to left and top to bottom. To avoid counting cells multiple times, the cells that were counted were marked clearly using the pencil tool of Paint software.

The data of all mitotic stages were statistically analyzed separately, and indexs were calculated using Microsoft Excel 2010. The differences in the tested treatments were analyzed using the LSD 1-way ANOVA SPSS 20 software, and statistical significance was set at  $P = 0.05$ .

Culcutating indexes:

(1) *Mitotic index - MI* was calculated as described by Njagi và Gopalan [16] và Journals [10]

$$MI (\%) = \frac{\text{Total number of cells at mitotic phases}}{\text{Total number of cells counted}} \times 100$$

(2) Mitotic inhibition was calculated as described by Journals [10]

Mitotic inhibition (%)

$$= \frac{\text{Mitotic Index in Control group} - \text{Mitotic Index in test groups}}{\text{Mitotic Index in control group}} \times 100$$

(3) Rate of deformed cells was calculated base on describe by Journals [10]

$$\text{Rate of deformed cells (\%)} = \frac{\text{Number of deformed cells}}{\text{Total number of cells counted}} \times 100$$

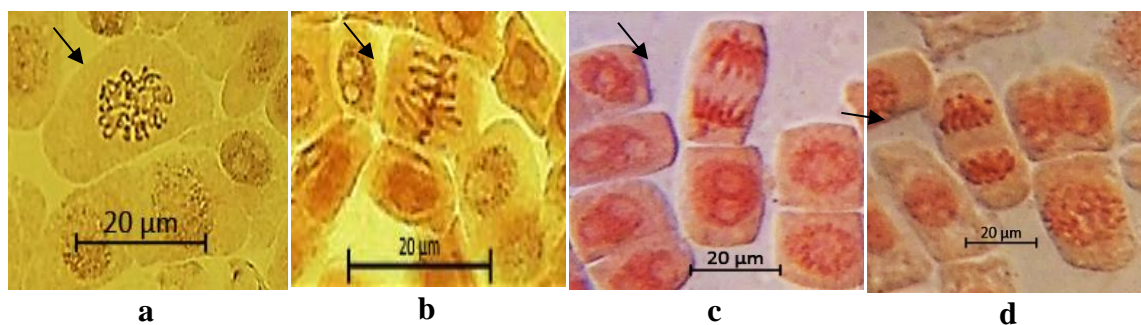
(4) Rate of disturbances cells was calculated base on describe by Journals [10]

Rate of disturbances cells (%)

$$= \frac{\text{Total number of disturbances cells at mitotic phases}}{\text{Total number of counted cells}} \times 100$$

### 3. Results and discussion

#### 3.1. Mitotic index of the root cells of *Allium fistulosum* L. after treatment with Altach 5EC



**Figure 1:** The images show root cells of *Allium fistulosum* L at different phases of mitosis at a zoom level of 200 X. **a:** prophase; **b:** metaphase; **c:** anaphase; **d:** telophase. Arrows: cells at the mitotic phase of figure a, b, c, and d

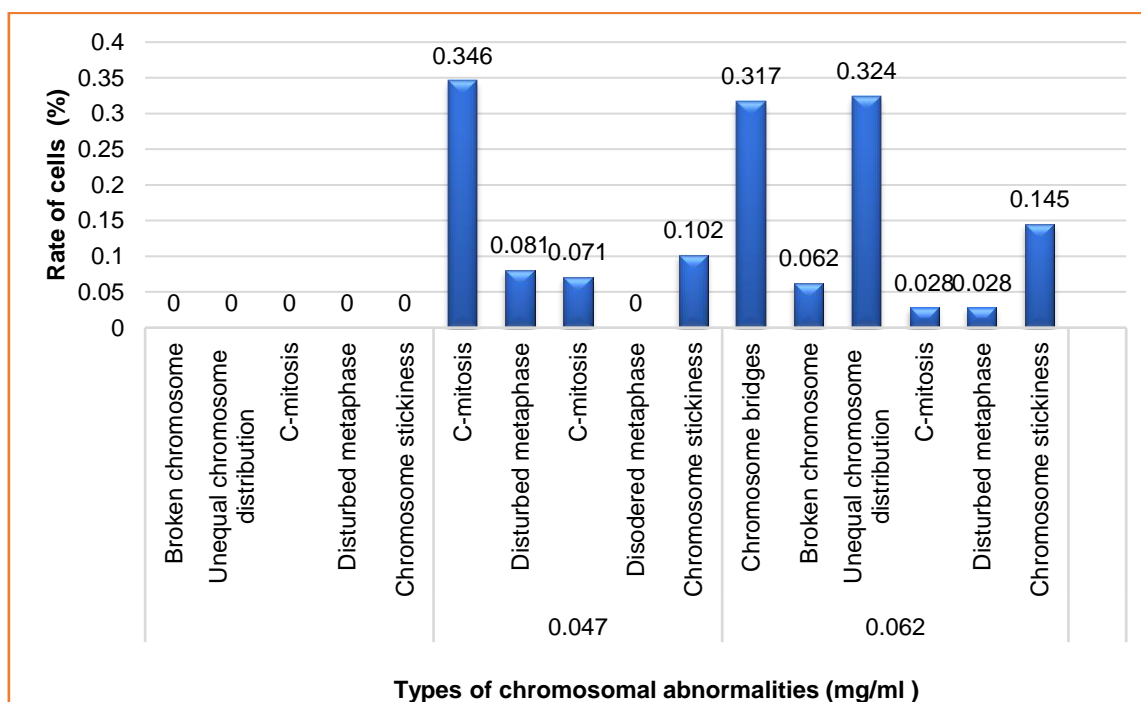
The number of dividing cells was counted in 1000 cells at prophase, metaphase, anaphase, and telophase (Figure 1) as described in the method section. Statistical analysis (Table 1) revealed a significant difference ( $P = 0.05$ ) between the indexes of the study samples at two concentrations. The mitotic index decreased in cells of samples treated with both concentrations of alpha-cypermethrin compare to the negative control, with a greater reduction observed at a concentration of 0.062 mg/ml compared to 0.047 mg/ml. This decrease may be due to the components present in the insecticide, particularly the active ingredient in the insecticide, which could have inhibited DNA replication during the S phase of interphase or prevented cells from entering mitosis [20]. Moreover, according to Bakare *et al.* [4], the decrease in the MI could also be attributed to DNA damage and genetic instability. LE [14] suggested that the MI could be reduced due to (1) the components present the insecticide inhibiting cell division, (2) abnormal spindle activity, and (3) the appearance of abnormal chromosomes.

Similar to this study, alpha-cypermethrin in the insecticide Fastac 100 EC also reduced the MI of human lymphocyte cells when treated with concentrations of alpha-cypermethrin lower than those used in this study (0.005, 0.01, 0.015, and 0.02 mg/ml) and longer treatment times (24 and 48 hours) [13]. This warns that there may be potential health effects associated with consuming products containing residual amounts of alpha-cypermethrin at concentrations as low as 0.005 mg/ml or higher.

**Table 1:** The mitotic index of root cells in *Allium fistulosum* L., statistically significant differences ( $P = 0.05$ ) when the samples were treated with Altach 5EC at concentrations of alpha-cypermethrin 0.047 mg/ml and 0.062 mg/ml for 8 hours

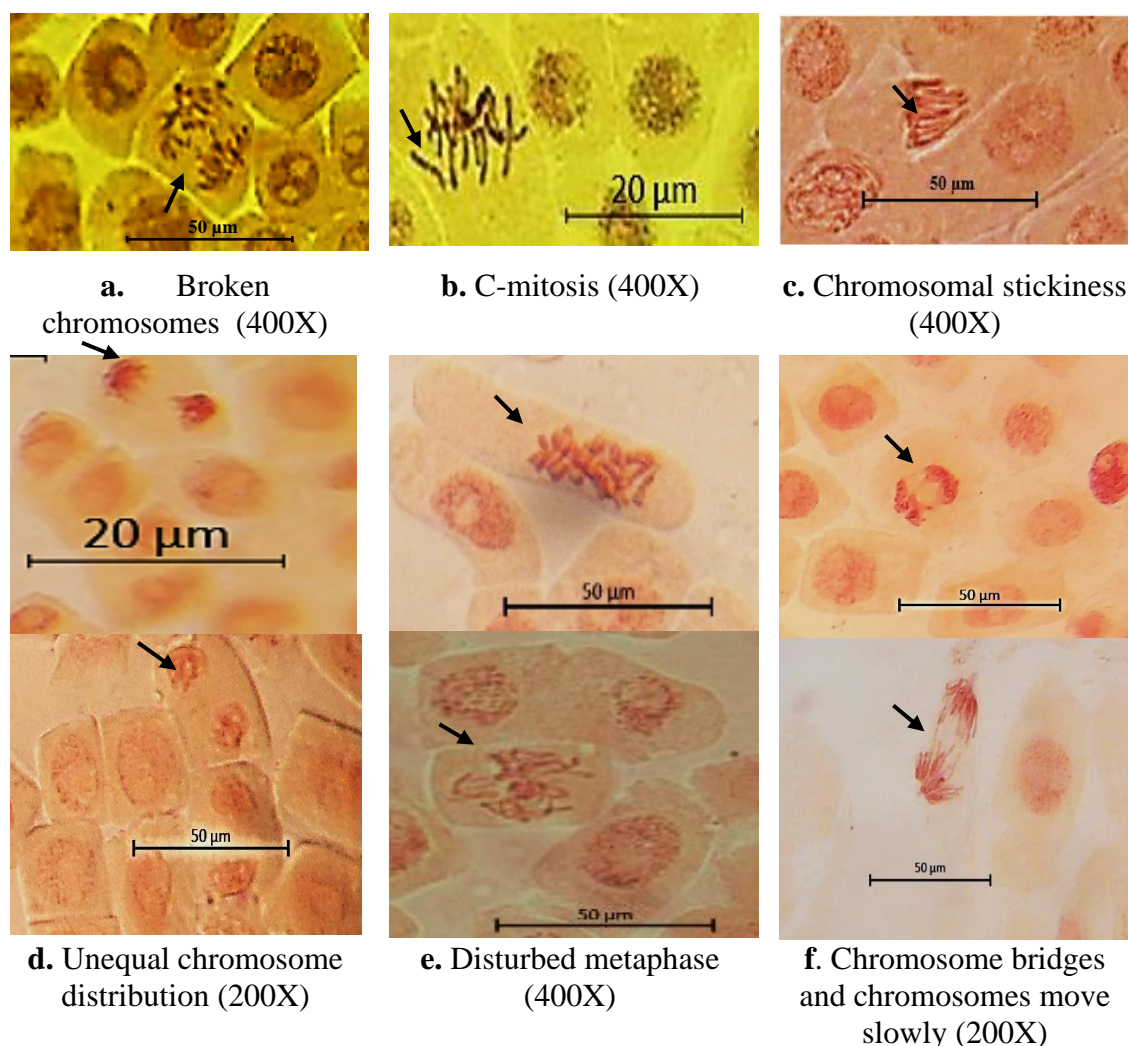
Concentration of alpha-cypermethrin	Total number of cells	Number of mitotic cells	MI (%)	Number of samples	Standard deviation	Rate of mitotic inhibition MI (%)
0.000	1000	232.000	23.20*	3	7.211	-
0.047	1000	214.000	21.40*	3	4.933	4.18
0.062	1000	182.333	18.23*	3	7.211	4.97

### 3.2. Chromosomal mutations and mitotic disturbances in root cells of *Allium fistulosum* L. after treatment with Altach 5EC



**Figure 2:** The effect of Altach 5EC containing alpha-cypermethrin at two concentrations of 0.047 mg/ml and 0.062 mg/ml on chromosomes and mitosis of root cells in *Allium fistulosum* L. during mitosis





**Figure 3:** Images of *Allium fistulosum* L. root cells with various types of chromosomal mutations and mitotic disturbances when treated with Attach 5EC at two concentrations of alpha-cypermethrin 0.047 mg/mL and 0.062 mg/mL. Arrows: the corresponding cells with chromosomal mutations or mitotic disturbances in images a, b, c, d, e, and f.

The Figure 2 indicate that various types of chromosomal mutations appeared at different rates, including the formation of chromosomal bridges and many cases resulting in slow movement and breakage of chromosomes. At the same time, several types of mitotic disorders were also observed, including fault polarization (which may lead to a decrease in the MI [14]), chromosomal stickiness (making it difficult for chromosomes to separate during cell division, leading to distortion and breakage), C-mitosis (mitotic cells that lack spindle fibres with unattached whole chromosomes lying scattered throughout the cell), and metaphase disorder (the sister chromosomes cannot be arranged on the same equatorial plane, affecting the uniform distribution of chromosomes to the two poles of the cell) (Figure 3). In which, the rate of cells with abnormal chromosomes during mitosis, such as chromosomal bridges at anaphase and telophase, broken chromosomes, and

chromosome stickiness, did not vary significantly between the two concentrations of the active ingredient. However, at the higher concentration of 0.062 mg/ml, the proportion of cells with fault polarization was much higher (0.324) than at the concentration of 0.042 mg/ml (0.071). This result is consistent with the MI (which is inversely proportional to the concentration of alpha-cypermethrin). Additionally, a certain proportion of cells with C-mitosis and metaphase disorder appeared only at the higher concentration of the active ingredient (Figure 2). These chromosomal abnormalities observed in this study have been reported in previous studies worldwide. In which, chromosomal stickiness (Figure 3c) may be caused by impact of the pesticide components on the physicochemical properties of DNA or protein, or both, leading to the formation of complexes with phosphate groups, making DNA condense or forming cross-linkages of the chromosomes [18]. In addition, the pesticide can also cause abnormal DNA condense, resulting in abnormal chromosome condense and entanglement of sister chromatids [3]; chromosomal breakages (Figure 3a) may be result of the formation of chromosomal bridges at anaphase. At the same time, the stickiness between chromosomes can also cause their breakage when they are stretched out during movement to the cell poles at anaphase, and chromosomes may move slowly [14]; the formation of chromosomal bridge at anaphase (Figure 3f) is one of the common types of chromosomal abnormalities observed when treating *Allium fistulosum* L. roots with Altach5EC at two different concentrations of alpha-cypermethrin. Chromosomal bridges may occur due to broken chromosomes and their stickiness [12]. In many instances, Altach 5EC have inhibited the formation of spindle fibers during cell division, resulting in mitotic cells lacking spindle fibers and causing whole unattached chromosomes to scatter throughout the cell (Figure 3b) [19].

Chromosomal mutations in cells due to the presence of commercial pesticides have been extensively studied in various plant species, especially *Allium cepa*. Asita and Mokhobo (2013) observed the appearance of cells with chromosomal stickiness, chromosomal bridges at telophase, broken chromosomes, micronuclei and C-mitosis when treating the roots of *Allium cepa* with four types of pesticides, QuickPhos (QP) (Aluminium Phosphide, 560 g/kg), Nuvan Profi (NP) (Dichlorvos, 124 g/kg), and Erioccephalus punctulatus plant smoke condensate (EPSC) at corresponding concentrations (mg/mL) of GT (12.5, 25, 50), QP (0.75, 1.5, 3.0), NP (0.064, 0.128, 0.256), EPSC (0.0025, 0.0049, 0.0098) for 24 hours. In all cases of chromosomal mutations, the percentage of cells with chromosomal stickiness accounted for up to 74.83%. Al-Ahmadi [18] found that treating the roots of *Allium cepa* with two insecticides, Kingbo and Azdar 10EC, at concentrations 0.625, 1.62, and 2.5 mL/L for 8, 16, and 24 hours led to the appearance of cells with chromosomal stickiness, C-mitosis, chromosome bridges at anaphase and telophase, and micronuclei. The higher the concentration and the longer the time of insecticide treatment, the higher the mutation rate. Srivastava [19] also reported that the roots of *Hordeum vulgare* L. Var. Karan 4 showed broken chromosomes, chromosome bridges, and small nuclei at interphase as well as chromosomes stickiness when treated with 0.05%, 0.1%, and 0.5% of insecticides, alphamethrin (AM), and monocrotophos (MP) for 6 hours. These finding suggest that many commercial insecticides have the potential to cause chromosomal mutations and mitotic disturbance in root cells of *Allium cepa*. These results also suggest that future studies could consider using different types of insecticides

in the same study to obtain more comprehensive and diverse data on the potential for causing chromosomal mutations and cell mitotic disturbances.

### 3. Conclusion

When treated with diluted Attach 5EC at concentrations of alpha-cypermethrin of 0.047 mg/ml and 0.062 mg/ml, the root cells of *Allium fistulosum* L. showed a tendency to decrease their mitotic index and caused various chromosomal mutations at a high rate. This leads to a greater probability of observing mutated chromosomes. At the same time, various types of mitotic disturbances were also observed, which can help to support and deepen students' knowledge of the practical lesson *Mitosis*. Therefore, Altach 5EC can be considered a suitable chemical mutagen for teaching "Chromosomal Structural Mutations" lesson within the Genetics-Evolution subject.

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## TÓM TẮT

### NGHIÊN CỨU SỬ DỤNG CHẾ PHẨM ALTACH 5EC XỬ LÝ RỄ HÀNH *ALLIUM FISTULOSUM* L. GÓP PHẦN NÂNG CAO HIỆU QUẢ BÀI THỰC HÀNH “ĐỘT BIẾN CẤU TRÚC NHIỄM SẮC THỂ” MÔN DI TRUYỀN - TIẾN HÓA TẠI KHOA SINH HỌC TRƯỜNG ĐẠI HỌC VINH

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Trong nghiên cứu này, chúng tôi đã sử dụng chế phẩm thương mại Altach 5EC để đánh giá sự ảnh hưởng của chế phẩm này đến nhiễm sắc thể và quá trình nguyên phân của tế bào rễ hành *Allium fistulosum* L. Kết quả cho thấy, trong thời gian 8 h được xử lý bằng dung dịch Altach 5EC chứa 0,047 mg/ml và 0,062 mg/ml hoạt chất alpha- cypermethrin, các mẫu nghiên cứu đều bị giảm chỉ số nguyên phân so với đối chứng âm. Đặc biệt, các dạng đột biến nhiễm sắc thể xuất hiện bao gồm: nhiễm sắc thể bị đứt gãy, hình thành cầu nhiễm sắc thể và nhiễm sắc thể di chuyển chậm. Ngoài ra, nghiên cứu cũng phát hiện nhiều dạng rối loạn phân bào nguyên nhiễm bổ sung tư liệu cho bài thực hành *phân bào nguyên nhiễm* như nhiễm sắc thể “lang thang”, rối loạn kỳ giữa, lệch trục và dính nhiễm sắc thể. Những kết quả này cho thấy Altach 5EC là chế phẩm thích hợp để sử dụng xử lý rễ hành phục vụ không những cho bài thực hành “Đột biến cấu trúc nhiễm sắc thể” mà cho cả bài “Phân bào nguyên nhiễm” thuộc môn học Di truyền - Tiến hoá tại khoa Sinh học, Trường Đại học Vinh

**Từ khóa:** *Allium fistulosum* L.; Altach 5EC; alpha cypermethrin; chỉ số nguyên phân; đột biến nhiễm sắc thể.